Cytochrome c (cyt c) displays a striking ability to perform many seemingly disparate functions within the cell. It is responsible for shuttling electrons between protein complexes in the mitochondria to generate ATP and is also a primary signal for apoptosis when improperly localized as a result of an interaction with the mitochondrial lipid cardiolipin (CL). We utilized reverse micelle nuclear magnetic resonance (RM-NMR) in order to investigate these different functional roles of cyt c at atomic resolution. The chemical shifts of RM encapsulated cyt c are essentially identical to the free solution protein, confirming structural fidelity. We have determined the structure of encapsulated cyt c to high resolution (0.45 Å backbone RMSD, 0.92 Å heavy atom RMSD) using standard solution NMR methods. Using pseudo-contact shifts (PCS), we find that the majority of the protein structure does not change significantly upon change in redox state. A subset of residues localized at the heme-exposed face of the protein undergo small structural changes upon change in redox state, localized to the binding site on cyt c for its BC1 complex partner. The interaction of cyt c with CL was investigated by titration of the lipid into the RM encapsulated protein. The confined space effect upon protein encapsulation in the RM allowed for separation and characterization of this peripheral interaction from the subsequent lipid insertion and unfolding of cyt c. These experiments provide the first detailed interface of the initial, largely electrostatic phase of the interaction.

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Prion Proteins and Mechanisms of Interaction with Model Membranes

Patricia Soto[1], William Graft[1], Roger Gonzalez[2], Chad Nieri[2], Bo Zhao[1], Jason C. Bartz[3].
[1]Physics, Creighton University, Omaha, NE, USA, [2]Biology, Creighton University, Omaha, NE, USA, [3]Medical Microbiology and Immunology, Creighton University, Omaha, NE, USA.

Prions are infectious agents responsible for transmissible spongiform encephalopathies, a fatal neurodegenerative disease in mammals, including humans. Prions propagate biological information by conversion of the nonpathological version of the prion protein, PrPc, to the infectious conformation, PrPSc. To shed light on the biogenesis of PrPSc on the cell surface, we will report on multiscale molecular modeling studies of enthalpy-driven binding modes of PrPc to model membranes and the conformational response of PrPc to such binding events. Our preliminary results suggest the existence of preferential binding spots on the PrPc surface driven by favorable protein-membrane electrostatics interactions. Upon binding, the conformational space of PrPc is reduced.

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Functional Characterization of Human Rhodopsin Mutations by Fluorescence Imaging

Caihong Jiang.
physics, east china normal university, Shanghai, China.

Rhodopsin is the membrane receptor responsible for photoreception in the vertebrate retina. Over 120 point mutations in rhodopsin are found to be related with autosomal dominant retinitis pigmentosa (ADRP) and the congenital stationary night blindness (CNSB). Despite of several mutations with intense studies, like P23H, a majority of rhodopsin mutations still need further investigations. In order to have extensive and quick functional characterization of these mutations, here we utilize fluorescence imaging to monitor rhodopsin cellular distribution, which reveals to us much useful information, like if rhodopsin has normal transportation to the cell membrane, interrrupted glycosylation or protein aggregates formation. The experiments are carried out through the following process: First, a series of human rhodopsin mutations were constructed, which include mutations responsible for both ADRP and CNSB, like G89D and G90D. Second, wild-type rhodopsin was expressed in 293S GnTi- cells with homogeneous N-glycosylation for protein detection, T-REx293 cells for glycosylation analysis, and Hela cells for immunofluorescence imaging seperately. Third, we also engineered a fusion protein rho-EGFP for in vivo study, with a green fluorescent protein insterted into human rhodopsin. We found that mutations that cause ADRP are usually misfolded and retained in endoplasmic reticulum and thus have low efficiency of 11-cis-retinal binding. And G90D mutant (causing CNSB) that was considered to have correct protein folding, however, showed an astonishing tendency to form inclusion bodies in our study, which may be related with its interrupted glycosylation in Golgi apparatus. In a word, we have established an effective and convenient system to investigate rhodopsin synthesis and transportation both in vitro and in vivo.

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Coexistence of Native-Like and Non-Native Misfolded Ferricytochrome C on the Surfac of Cardiolipin Containing Liposomes

Leah A. Pandiscia, Reinhard Schweitzer-Stenner.
Chemistry, Drexel University, Philadelphia, PA, USA.

Cytochrome c, in spite of adopting a rather rigid structure around its prosthetic heme group, is rather diverse with regard to its function and structural variability. On the surface of the inner membrane of mitochondria it serves as an electron transfer carrier. However, at conditions, which have not yet been unambiguously identified, it can adopt a variety of non-native conformations some of which exhibit peroxidase activity. Cardiolipin-containing liposomes have served as ideal model system to investigate the various modes of interaction between cytochrome c and the inner mitochondrial membrane. We probed the binding of horse heart ferricytochrome to liposomes formed with 20% tetraoleoyl cardiolipin and 80% dioleoyl-sn-glycero-3-phosphocholine as a function of lipid/protein ratio by fluorescence, fluorescence anisotropy, and visible circular dichroism spectroscopy. A global analysis of our data revealed the existence of three binding sites on the protein which causes rather different degrees of protein unfolding. We found that two of the three modes of interaction between protein and liposome led to conformational changes. A more native-like state or a higher population of the native state is obtained in the presence of NaCl, which also leads to a nearly total inhibition of the binding via the two lower affinity protein binding sites. Our results can be rationalized interms of the two state equilibrium between a compact C and an extended E-state proposed by Pletneva and coworkers. We conjecture that the bound state produced by the high affinity site 1 binding might bear the closest relationship to the protein which functions as electron carrier in the mitochondria. The higher E-state population produced by site 2 and 3 binding is likely to increase the protein's capability to function as a peroxidase.

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Neisserial Opa Protein Dynamics and Interaction with Host Ceacam Receptors

Marissa K. Kieber, Tsega Solomon, Linda Columbus.
Department of Chemistry, University of Virginia, Charlottesville, VA, USA.

Human pathogens *Neisseria gonorrhoeae* and *N. meningitides* are unique in their utilization of opacity-associated (Opa) proteins to mediate bacterial uptake into non-phagocytic cells. Opa proteins engage either heparan sulfate proteoglycan (HSPG) receptors or carcinoembryonic antigen-related cellular adhesion molecules (CEACAMs) to hijack host cellular mechanisms, which induces bacterial engulfment. The Opa family of proteins are eight stranded β-barrels with four extracellular loops. Regions in loops two and three contain hypervariable sequences among Opa variants and dictate receptor specificity. We aim to investigate the structural determinants of Opa-receptor interactions. Overall loop dynamics of Opa_{60}, a CEACAM-binding Opa variant, were determined using CW-EPR and combined with the limited NMR relaxation data. Results indicate that the loops and hypervariable regions are highly mobile on the nanosecond timescale. Initial DEER experiments measured distances between Opa_{60} and CEACAM in the complex and preliminary models consistent with these distances will be presented. Determining the interactions between Opa and CEACAM will provide an understanding of the molecular interactions that mediate the entry of a foreign body into non-phagocytic cells.

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Membrane Protein Misfolding Enforces the Positive-Inside Rule

Nicholas B. Last, Christopher Miller.
Brandeis Univ./HHMI, Waltham, MA, USA.

Inner membrane proteins have long been known to follow the ''positive-inside rule'', where cytoplasmic loops tend to have a greater number of cationic residues than periplasmic or extracellular loops. This effect can be seen dramatically in dual-topology proteins, such as the small multidrug transporter EmrE, where relatively balanced charge between the protein faces leads to mixed insertion in the membrane; some molecules are inserted with a cytoplasmic N-terminus, and some with a periplasmic. Addition or removal of positive charge can bias the orientation of the inserted protein. Surprisingly, this is true even if the mutation does not occur until after the synthesis of several transmembrane helices. Here, we examine how positive charges bring about orientation bias. We use a GFP-based assay to examine both the orientation of the protein as well as rates of misinsertion and degradation. We find a significant pool of degraded or misfolded protein to be present even with wild-type EmrE. In addition to the redistribution of well-folded protein, the amount, type, and orientation of degradation products changes